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Biochemica and Biophysica Acta (1985) 824 pp.233-242

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(54) 2'-fluoro-2'-deoxy-cytidine for anti-viral use

(57) The present invention provides a 2'-fluoro-2'-deoxy-ribonucleoside and salts thereof for use in the treatment or prophylaxis of an influenza virus infection.

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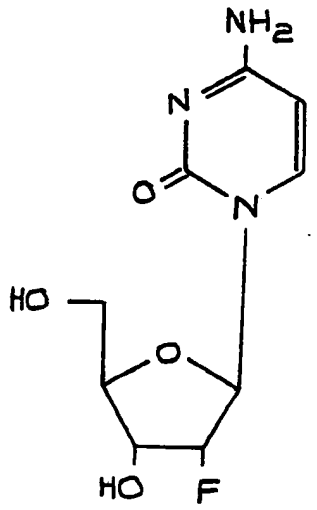
ANTIVIRAL COMPOUNDS

The present invention relates to a 2'deoxy-2'-fluoro-ribonucleoside for use in a method of treatment of the human or animal body by therapy.

In particular, the invention provides 2'-fluoro-2'deoxy-cytidine (dCfl) of the formula (I):



(I)

for the treatment or prophylaxis of influenza virus infections.

It has been shown that in vitro dCfl has an inhibitory effect against herpes simplex virus I and II, Pseudorabies virus, and equine abortion virus. (F.Wohlras et al, Biochemica et Biophysica Acta, 824,233-242-(1985)).

We have now surprisingly found that dCfl is highly effective against influenza A and B virus.

The present invention thus also provides:

a) a method for the treatment or prophylaxis of an influenza virus infection in a mammal including man, which comprises treating a subject with an effective non-toxic amount of dCfl, and

b) use of dCfl in the manufacture of a medicament for the treatment or prophylaxis of an influenza virus infection.

Depending on the process conditions and the starting materials, the compound of the formula (I) is obtained either as the free base or as a salt. Both the free base and the salts of the end product are included within the scope of the invention. Thus, basic, neutral or mixed salts may be obtained as well as hemi, mono, sesqui or polyhydrates. Acid addition salts of the new compound may in a manner known per se be transformed into free base using basic agents such as alkali or by ion exchange. The free bases obtained may also form salts with organic or inorganic acids.

Salts of the compound of the formula I which may be conveniently used in therapy include physiologically acceptable base salts, e.g. derived from an appropriate base, such as alkali metal (e.g. sodium), alkaline earth metal (e.g. magnesium) salts, ammonium and NX_4 (wherein X is C_{1-4} alkyl) salts.

Hereafter, dCfl and its salts are also referred to as the 'compound of the invention', and in relation to formulations containing it, the 'active ingredient'.

dCfl may be synthesized -by- any known method, for example from 2,2'-O-anhydrocytidine (cyclocytidine), as described by R. Mengel and W. Guschlbauer, Angew Chemie Intl. Ed. 17,525(1978).

The compound of the invention may be administered to recipients such as mammals including humans by any route appropriate to the condition to be treated, suitable routes including oral, pulmonic, rectal, nasal, topical (including buccal and sublingual), vaginal and parenteral (including subcutaneous, intramuscular, intravenous, intradermal, intrathecal and epidural). It will be appreciated that the preferred route may vary with, for example, the condition of the recipient.

The amount required of the active ingredient for the treatment of influenza virus infections will depend upon a number of factors including the severity of the condition to be treated and the identity of the recipient and will ultimately be at the discretion of the attendant physician. In general, however, for each of these utilities and indications, a suitable, effective dose will be in the range 0.1 to 100 mg per kilogram body weight of recipient per day, preferably in the range 5 to 30 mg per kilogram body weight per day and most preferably in the range 10 to 20 mg per kilogram body weight per day; an optimum dose is about 15 mg per kilogram body weight per day (unless otherwise indicated all weights of active ingredient are calculated as a compound of formula I; for salts thereof the figures would be increased proportionately.) The effective dose may optionally be presented as two, three, four or more sub-doses administered at appropriate intervals throughout the day. These sub-doses may be administered in unit dosage forms, for example, containing 1 to 2000 mg, preferably 20 to 500 mg and most preferably 100 to 400 mg of active ingredient per unit dosage form.

The compound of the present invention may be administered alone or in combination with other therapeutic agents, for example, with amantidine or with ribavirin, which are known anti-influenza agents, or any other agents which when in combination with a compound according to the invention provide a beneficial therapeutic effect. While it is possible for the compound to be administered alone it is preferable to present it as a pharmaceutical formulation. The formulations of the present invention comprise at least one active ingredient, as above defined, together with one or more acceptable carriers thereof and optionally other therapeutic ingredients. The carrier(s) must be "acceptable" in the sense of being compatible with the other ingredients of the formulation and not deleterious to the recipients thereof.

The formulations include those suitable for oral, pulmonic, rectal, nasal, topical (including buccal and sublingual), vaginal or

parenteral (including subcutaneous, intramuscular, intravenous, intradermal, intrathecal and epidural) administration. The formulations may conveniently be presented in unit dosage form and may be prepared by any of the methods well known in the art of pharmacy. Such methods include the step of bringing into association the active ingredient with the carrier which constitutes one or more accessory ingredients. In general the formulations are prepared by uniformly and intimately bringing into association the active ingredient with liquid carriers or finely divided solid carriers or both, and then, if necessary, shaping the product.

Formulations of the present invention suitable for oral administration may be presented as discrete units such as capsules, cachets or tablets each containing a predetermined amount of the active ingredient; as a powder or granules; as a solution or a suspension in an aqueous liquid or a non-aqueous liquid; or as an oil-in-water liquid emulsion or a water-in-oil liquid emulsion. The active ingredient may also be presented as a bolus, or paste or may be contained within liposomes.

A tablet may be made by compression or moulding, optionally with one or more accessory ingredients. Compressed tablets may be prepared by compressing in a suitable machine the active ingredient in a free-flowing form such as a powder or granules, optionally mixed with a binder (e.g. povidone, gelatin, hydroxypropylmethyl cellulose), lubricant, inert diluent, preservative, disintegrant (e.g. sodium starch glycolate, cross-linked povidone, cross-linked sodium carboxymethyl cellulose), surface-active or dispersing agent. Moulded tablets may be made by moulding in a suitable machine a mixture of the powdered compound moistened with an inert liquid diluent. The tablets may optionally be coated or scored and may be formulated so as to provide slow or controlled release of the active ingredient therein using, for example, hydroxpropylmethylcellulose in varying proportions to provide the desired release profile.

A capsule may be made by filling a loose or compressed powder on an appropriate filling machine, optionally with one or more additives. Examples of suitable additives include binders such as povidone, gelatin, lubricants, inert diluents, distintegrants as for tablets. Capsules may also be formulated to contain pellets or discrete sub-units to provide slow or controlled release of the outline ingredient. This can be achieved by extruding and spheronising a wet mixture of the drug plus an extrusion aid (e.g. microcrystalline cellulose) plus a diluent such as lactose. The spheroids thus produced can be coated with a semi-permeable membrane (e.g. ethyl cellulose, Eudragit WE30D) to produce sustained release properties.

For topical administration the formulations are preferably applied as an ointment or cream containing the active ingredient in an amount of, for example, 0.075 to 20% w/w, preferably 0.2 to 15% w/w and most preferably 0.5 to 10% w/w. When formulated in an ointment, the active ingredients may be employed with either a paraffinic or a water-miscible ointment base. Alternatively, the active ingredients may be formulated in a cream with an oil-in-water cream base or as in a water in oil base.

If desired, the aqueous phase of the cream base may include, for example, at least 30% w/w of a polyhydric alcohol, i.e. an alcohol having two or more hydroxyl groups such as propylene glycol, butane-1,3-diol, mannitol, sorbitol, glycerol and polyethylene glycol and mixtures thereof. The topical formulations may desirably include a compound which enhances absorption or penetration of the active ingredient through the skin or other affected areas. Examples of such dermal penetration enhancers include dimethylsulphoxide and related analogues.

The oily phase of the emulsions of this invention may be constituted from known ingredients in a known manner. While this phase may comprise merely an emulsifier (otherwise known as an emulgent), it desirably comprises a mixture of at least one emulsifier with a fat or

an oil or with both a fat and an oil. Preferably, a hydrophilic emulsifier is included together with a lipophilic emulsifier which acts as a stabilizer. It is also preferred to include both an oil and a fat. Together, the emulsifier(s) with or without stabilizer(s) make up the so-called emulsifying wax, and the wax together with the oil and/or fat make up the so-called emulsifying ointment base which forms the oily dispersed phase of the cream formulations.

Emulgents and emulsion stabilizers suitable for use in the formulation of the present invention include Tween 60, Span 80, cetostearyl alcohol, myristyl alcohol, glycerol mono-stearate and sodium lauryl sulphate.

The choice of suitable oils or fats for the formulation is based on achieving the desired cosmetic properties, since the solubility of the active compound in most oils likely to be used in pharmaceutical emulsion formulations is very low. Thus the cream should preferably be a non-greasy, non-staining and washable product with suitable consistency to avoid leakage from tubes or other containers. Straight or branched chain, mono- or dibasic alkyl esters such as di-isoadipate, isocetyl stearate, propylene glycol diester of coconut fatty acids, isopropyl myristate, decyl oleate, isopropyl palmitate, butyl stearate, 2-ethylhexyl palmitate or a blend of branched chain esters known as Crodamol-CAP may be used, the last three being preferred esters. These may be used alone or in combination depending on the properties required. Alternatively, high melting point lipids such as white soft paraffin and/or liquid paraffin or other mineral oil can be used.

Formulations suitable for topical administration to the eye also include eye drops wherein the active ingredient is dissolved or suspended in a suitable carrier, especially an aqueous solvent for the active ingredient. The active ingredient is preferably present in such formulations in a concentration of 0.5 to 20%, advantageously 0.5 to 10% particularly about 1.5% w/w.

Formulations suitable for topical administration in the mouth include lozenges comprising the active ingredient in a flavoured basis, usually sucrose and acacia or tragacanth; pastilles comprising the active ingredient in an inert basis such as gelatin and glycerin, or sucrose and acacia; and mouth-washes comprising the active ingredient in a suitable liquid carrier.

Formulations for rectal administration may be presented as a suppository with a suitable base comprising for example cocoa butter or higher fatty alcohol (e.g. hard wax European Pharmacopeia) or triglycerides and saturated fatty acids (e.g. Witepsol).

Formulations suitable for nasal administration wherein the carrier is a solid include a coarse powder having a particle size for example in the range 20 to 500 microns which is administered in the manner in which snuff is taken, i.e. by rapid inhalation through the nasal passage from a container of the powder held close up to the nose. Suitable formulations wherein the carrier is a liquid, for administration as for example a nasal spray or as nasal drops, include aqueous or oily solutions of the active ingredient.

Suitable formulations for administration by inhalation include fine particle dusts or mists which may be generated by means of various types of metered dose- pressurised aerosols, nebulizers or insufflators.

For pulmonary administration via the mouth, the particle size of the powder or droplets is typically in the range 0.5 - 10μm, preferably 1 - 5μm, to ensure delivery into the bronchial tree. For nasal administration, a particle size in the range 10 - 500μm is preferred to ensure retention in the nasal cavity.

Metered dose inhalers are pressurised aerosol dispensers, typically containing a suspension or solution formulation of the active ingredient in liquefied propellant. During use these devices discharge

the formulation through a valve adapted to deliver a metered volume, typically from 10 - 150μl, to produce a fine particle spray containing the active ingredient. Suitable propellants include propane and butane and mixtures thereof. The formulation may additionally contain co-solvents, for example ethanol, surfactants such as oleic acid or sorbitan trioleate, antioxidants and/or suitable flavouring agents.

Nebulizers are commercially available devices that transform solutions or suspensions of the active ingredient into an aerosol therapeutic mist either by means of acceleration of a compressed gas through a narrow venturi orifice, typically air or oxygen, or by means of ultrasonic agitation. Suitable formulations for use in nebulizers consist of the active ingredient in a liquid carrier and comprising up to 40%w/w of the formulation, preferably less than 20%w/w. The carrier is typically water or a dilute aqueous alcoholic solution, preferably made isotonic with body fluids by the addition of, for example, sodium chloride. Optional additives include preservatives if the formulation is not prepared sterile, for example methylhydroxybenzoate, antioxidants, flavouring agents, volatile oils, buffering agents and surfactants.

Suitable formulations for administration by insufflation include finely comminuted powders which may be delivered by means of an insufflator or taken into the nasal cavity in the manner of a snuff. In the insufflator, the powder is contained in capsules or cartridges, typically made of gelatin or plastic, which are either pierced or opened in-situ and the powder either presented to air drawn through the device upon inhalation or alternatively delivered by means of a manually operated pump. The powder employed in the insufflator consists either solely of the active ingredient or of a powder blend comprising the active ingredient, a suitable powder diluent such as lactose, and an optional surfactant. The active ingredient typically comprises from 0.1 - 100%w/w of the formulation.

Pressurised aerosol formulations for inhalation are preferably arranged so that each metered dose contains from 0.05 to 5 mg of a compound of the invention. Similarly, powder formulations for insufflations are so arranged that each unit dose contains from 0.5 to 50 mg. Solution or suspension formulations for nebulisation are arranged as to deliver doses between 1 and 1500 mg. The compound of the invention may be administered by these devices once or several times daily, up to several doses, for example three or four, being given on each occasion.

Formulations suitable for vaginal administration may be presented as pessaries, tampons, creams, gels, pastes, foams or spray formulations containing in addition to the active ingredient such carriers as are known in the art to be appropriate.

Formulations suitable for parenteral administration include aqueous and non-aqueous sterile injection solutions which may contain anti-oxidants, buffers, bacteriostats and solutes which render the formulation isotonic with the blood of the intended recipient; and aqueous and non-aqueous sterile suspensions which may include suspending agents and thickening agents, and liposomes or other microparticulate systems which are designed to target the compound to blood components or one or more organs. The formulations may be presented in unit-dose or-multi-dose containers, for example sealed ampoules and vials, and may be stored in a freeze-dried (lyophilized) condition requiring only the addition of the sterile liquid carrier, for example water for injections, immediately prior to use. Injection solutions and suspensions may be prepared extemporaneously from sterile powders, granules and tablets of the kind previously described.

Preferred unit dosage formulations are those containing a daily dose or unit, daily sub-dose, as herein above recited, or an appropriate fraction thereof, of an active ingredient.

It should be understood that in addition to the ingredients particularly mentioned above the formulations of this invention may include other agents conventional in the art having regard to the type of formulation in question, for example those suitable for oral administration may include flavouring agents.

EXAMPLE 1

Preparation of 2'Deoxy-2'-fluorocytidine

A mixture of 18-crown-6 (25g), potassium fluoride (11g), DMF (dimethylformamide) (1800ml) and toluene (1800ml), fitted with a Dean-Stark trap under N_2, was bought to reflux and the solvents were removed azeotropically until the temperature reached 173°C. The solution was cooled to 120°C, O2,2'-anhydrocytidine hydrochloride (33g) added, and the mixture stirred at 120°C for 4 1/2 hours.

After cooling, the solvents were removed in vacuo, the residue stirred with MeOH(700ml) and filtered. The filtrate was evaporated, absorbed onto SiO_2 (80g) and placed onto a silica column. Elution with $CHCl_3$/MeOH (9:1), (6:1) and finally (4:1) gave 6.50g (21%) of the title compound after crystallization from water.

M.pt. 165-168°C. (Softens-at- 100°C)
Anal. $C_9H_{11}FN_3O_4 2H_2O$ requires C, 38.57;H,5.39;N,15.00
Found C, 38.50;H,5.77;N,14.94

The compound of the invention was used an in vitro assay to test its activity against influenza. A plaque reduction assay (Collins and Bauer, 1977) was carried out using MDCK (Madin Darby Canine Kidney) cells with trypsin in the overlay to aid plaque formation (Appleyard and Maber, 1974;Hayden etal, 1980).

The results are given in Table I below:

TABLE I

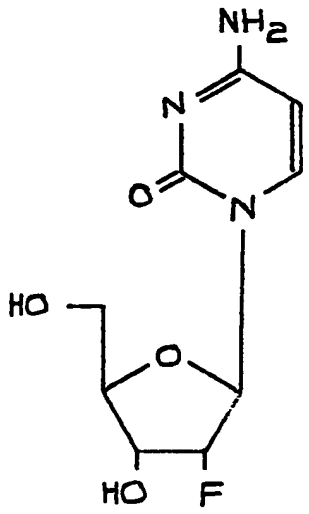
Anti-influenza Activity of dCfl

MDCK cells IC_{50} μM
1-5

References Appleyard,G and Maber,H.B, Plaque Formation by Influenza Vi-ruses in the Presence of Trypsin. J.Gen.Virol.25, 351-357.(1974).
Collins,P.and Bauer,D.J, Relative Potencies of Anti-Herpes Compounds. Ann.N.Y.Acad.Sci.284, 49-59,(1977).
Hayden,F.G,Cotek,M. and Douglas,R.G.,Plaque Inhibition Assay for Drug Susceptibility Testing of Influenza Viruses. Antimicro Agents and Chemo.17, 865-870.(1980).

CLAIMS

1. Use of 2'-deoxy-2'fluoro-cytidine, which compound has the formula (I)



(I)

or a salt thereof, in the manufacture of a medicament for the treatment or prophylaxis of an influenza virus infection.

2. A pharmaceutical formulation comprising as active ingredient a compound as claimed in Claim 1, together with at least one pharmaceutically acceptable carrier therefor.

3. A pharmaceutical formulation comprising as active ingredient a compound as claimed in Claim 1, together with at least one pharmaceutically acceptable carrier therefor for use in the treatment of influenza virus infections.

LL/JJ/10th August, 1990.

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